Prior Art evidence in support of enablement and written disclosure

D1:	Am J Cardiol. 1991 Mar 15;67(7):569-72. **Long-term follow-up of the first 56 patients treated with intracoronary self-expanding stents (the Lausanne experience).** Goy JJ, Sigwart U, Vogt P, Stauffer JC, Kaufmann U, Urban P, Kappenberger L. Department of Internal Medicine, University Hospital Lausanne, Switzerland. Fifty-six patients treated with the **self-expanding intracoronary stent for acute occlusion during percutaneous transluminal coronary angioplasty (PTCA) or restenosis** were followed for 24 to 43 months (mean 34). **Successful deployment and positioning were achieved in 55 of 56 patients**. Occlusion of the stent was documented in 8 patients, the earliest occurring 30 minutes and the latest 8 months after implantation. Three of the occluded stents were recanalized by PTCA. Coronary artery bypass grafts (CABG) were required in 4 patients: 1 for symptomatic restenosis, 1 for left main stenosis adjacent to the stent and 2 for acute ischemia during the in-hospital stay (less than 7 days). Myocardial infarction occurred in the territory of the stented vessel in 8 patients. Seven patients died between 1 day and 19 months after implantation. Local bleeding complications occurred in 10 patients, with 5 requiring blood transfusion. Restenosis within the stent was angiographically documented in 5 patients (9%). A new lesion in the treated vessel was found in 10 patients, followed by implantation of a second stent in 5 and a third stent in 1 patient. Medical treatment was instituted in the remaining 4 patients. Forty-nine patients (88%) are alive. Twenty-nine patients (51%) remained asymptomatic, and 44 (78%) are in a better functional class than before the implantation. Eleven of 15 (79%) major complications (acute occlusions or deaths) occurred in patients who received a stent in the **left anterior descending coronary artery**. In conclusion, **implantation of the self-expanding intracoronary stent appears to be a new therapeutic option for treating acute occlusion or restenosis after PTCA**.
D2:	J Interv Cardiol. 2001 Aug;14(4):439-42. **Ostial left anterior descending coronary artery stent positioning: partial preinflation prevents stent oscillation and facilitates accurate deployment.** Hildick-Smith DJ, Shapiro LM. Department of Cardiology, Papworth Hospital, Cambridgeshire, CB3 8RE, United Kingdom. david.hildick-smith@papworth-tr.anglox.nhs.uk Ostial intracoronary stent deployment is complicated by the influence of cardiac motion, which causes the stent to oscillate back and forth during the cardiac cycle. Accurate deployment can be facilitated by initial low pressure inflation of the balloon on which the stent is mounted. **This stabilizes the stent within the stenosis, while still allowing adjustment of the exact stent location prior to deployment**.
D3:	Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, Volume 1436, Issue 3, 4 January 1999, Pages 577-584. **Effect of increased afterload on cardiac lipoprotein lipase and VLDL receptor expression** N. D. Vaziri, K. Liang and C. H. Barton Received 5 October 1998; accepted 4 November 1998; available online 26 February 1999. Fatty acids are a major source of fuel for energy production by myocytes. Lipoprotein lipase (LPL) and very low density lipoprotein (VLDL) receptor are abundantly expressed by the heart and skeletal muscles. **LPL and possibly VLDL receptor represent the primary route of access to fatty acids contained in circulating triglyceride-rich lipoproteins**. Physical exercise and thyroid hormone, which promote energy consumption, upregulate LPL expression in skeletal muscles. This study tested the hypothesis that increased cardiac workload might modulate myocardial LPL and/or VLDL receptor expressions. Accordingly, cardiac tissue LPL activity, LPL and VLDL receptor proteins and mRNA abundance were studied in Sprague–Dawley rats 4 weeks after induction of severe thoracic aorta constriction or sham operation. Elevation of afterload with thoracic aortic constriction led to a significant cardiomegaly and a marked upregulation of cardiac LPL activity, LPL mRNA and LPL protein abundance, but did not modify VLDL receptor mRNA or protein abundance. Thus, increased cardiac workload in this model results in upregulation of myocardial LPL expression which can enhance fatty acid availability to accommodate the heart's increased energy requirement.

D4:	Journal of Huazhong University of Science and Technology -- Medical Sciences --, Huazhong University of Science and Technology, Vol. 11, Number 1 / March 1991, p. 39-44. **Relationship of VLDL receptor and LPL in metabolism of VLDL by macrophage** Deng Yao-zu , Feng Zong-chen, Wang Hong-xing, Jiang Wei-guo, Zhong Yi-qiang and Wang Cai-fu Received: 23 March 1990 Summary Macrophages were incubated with ^{125}I-VLDL for 5 h in presence or absence of lipoprotein lipase (LPL) inhibitor, benzene boronic acid (BBA). Both the uptake and degradation of ^{125}I-VLDL by macrophages were saturable, and the uptake and degradation curves were virtually identical. When macrophages were incubated with ^{125}I-VLDL for 10 h in presence of BBA, the uptake and degradation of ^{125}I-VLDL were still saturable. However, in absence of BBA, the uptake and degradation were no longer saturable. **The results suggest that with macrophages incubated with VLDL for a shorter period, VLDL was taken up predominantly via receptor pathway, with a longer period of incubation, LPL played a striking role in uptake of VLDL**.
D5:	Nature Genetics 18, 325 - 330 (1998) **Human gene targeting by viral vectors** David W. Russell & Roll K. Hirata Stable transduction of mammalian cells typically involves random integration of viral vectors by non-homologous recombination. Here we report that vectors based on adeno-associated virus (AAV) can efficiently modify homologous human chromosomal target sequences. Both integrated neomycin phosphotransferase genes and the hypoxanthine phosphoribosyltransferase gene were targeted by AAV vectors. Site-specific genetic modifications could be introduced into approximately 1% of cells, with the highest targeting rates occurring in normal human fibroblasts. These results suggest that AAV vectors could be used to introduce specific genetic changes into the genomic DNA of a wide variety of mammalian cells, including therapeutic gene targeting applications.
D6:	Hum Gene Ther. 1995 Jul;6(7):853-63. **Retroviral-mediated gene transfer and expression of human lipoprotein lipase in somatic cells.** Lewis ME, Forsythe IJ, Marth JD, Brunzell JD, Hayden MR, Humphries RK. Department of Medical Genetics, University of British Columbia, Vancouver, Canada. Lipoprotein lipase (LPL) is an enzyme responsible for the hydrolysis of triglyceride-rich circulating lipoproteins. Humans with complete defects in LPL activity present from infancy with failure to thrive, eruptive xanthomas, pancreatitis, and lactescent plasma. In addition, heterozygous carriers for this disorder may be at increased risk for the development of coronary artery disease. In view of a potential strategy for correcting complete or partial LPL deficiency, **a 1.56-kb human LPL cDNA was inserted into a series of recombinant myeloproliferative sarcoma virus (MPSV)-based retroviral vectors under transcriptional control of the constitutive MPSV long terminal repeat (LTR). Stable gene transfer and enhanced expression of human LPL was observed at both the RNA and protein level in a variety of somatic cell types in vitro**. Genetically modified cell populations included mouse NIH-3T3 fibroblasts and C_2C_{12} myoblasts, primary human fibroblasts, and established human hematopoietic cell lines of erythroid (K562), myelocytic (HL60), and monocytic (U937,THP-1) type. The achieved levels of bioactive human LPL were found to vary widely between the different transduced cell lines, which may be critical to an approach to gene therapy. Transduced primary human fibroblasts yielded maximal elevation of LPL immunoreactive mass and activity of at least 24- and 50-fold, respectively, above constitutively expressed levels for this cell type. Human fibroblasts, therefore, appear to accommodate in vitro the complex processes readily leading to the maturation and secretion of bioactive human LPL and may serve as an effective cellular vehicle for LPL gene delivery and expression in human LPL deficiency.

D7:	Biochemical and Biophysical Research Communications, Volume 270, Issue 3, 21 April 2000, Pages 997-1001 **Increased Intracellular Triglyceride in C_2C_{12} Muscle Cells Transfected with Human Lipoprotein Lipase** Paul Poirier, Tere Marcell, Patricia Uelmen Huey, Isabel R. Schlaepfer, Geoffrey C. Owens, Dalan R. Jensen and Robert H. Eckel, Received 9 March 2000. Available online 26 March 2002. Abstract Much of the knowledge about the cell biology of lipoprotein lipase (LPL) in vitro has been gained from adipose tissue model systems. However, the importance of skeletal muscle lipoprotein lipase (SMLPL) to both lipoprotein and muscle metabolism remains unclear. Although the production of LPL in cultured myocytes has been documented, the amount of enzyme activity produced is small. To develop a more suitable tissue culture model for SMLPL, **mouse C_2C_{12} myoblasts were stably transduced with a retroviral vector encoding the full-length human LPL (hLPL) cDNA**. Control cells were transduced with a vector encoding ß-galactosidase. LPL expression was assayed as a function of cell growth by measuring LPL activity on days 3, 7, 9, 11, and 14 after subculture. The hLPL-transduced myoblasts increasingly overexpressed both heparin-releasable (HR) and intracellular (IN) LPL activity compared to nontransduced myoblasts ($P < 0.001$ at Day 11) and myoblasts transduced with the control vector ($P < 0.001$ at Day 11). This increase occurred while LPL mRNA levels remained stable between days 3 and 14. As expected, IN LPL activity was also increased in the transduced cells. High levels of LPL activity were also obtained after differentiating the C_2C_{12} cells into myotubes by serum deprivation. Additionally, throughout the time course, C_2/LPL cells had greater amounts of intracellular triglyceride than both the C_2C_{12} and the C_2/ß-GEO cells ($P = 0.005$ and $P < 0.001$, respectively) with the largest differences seen on day 14 of the time course ($P = 0.001$, C_2/LPL vs C_2C_{12} r or C_2/ß-GEO cells). Thus, C_2C_{12} myoblasts stably transduced with hLPL markedly overexpressed both HR and IN LPL activity compared to control cells which, in turn, was associated with increases in intracellular triglyceride content. Because LPL regulation in tissues is mostly posttranslational, this new in vitro model will permit the in-depth study of the posttranslational regulation of SMLPL and provide new insights into the fate of lipoprotein-derived fatty acids in muscle.
D8:	Eur J Clin Invest. 2001 Dec;31(12):1040-7. **Food deprivation increases post-heparin lipoprotein lipase activity in humans.** Ruge T, Svensson A, Eriksson JW, Olivecrona T, Olivecrona G. Department of Medical Biosciences, Umeå University, SE-901 07 Umeå, Sweden. OBJECTIVE: To study the effect of fasting on lipoprotein lipase (LPL) activity in human post-heparin plasma, representing the functional pool of LPL. DESIGN: Fourteen healthy volunteers were recruited for the study. The subjects were fasted for 30 h. Activities of LPL and hepatic lipase (HL), and LPL mass, were measured in pre- and post-heparin plasma in the fed and in the fasted states, respectively. For comparison, LPL and HL activities were measured in pre- and post-heparin plasma from fed and 24-h-fasted guinea pigs. RESULTS: Fasting caused a significant drop in the levels of serum insulin, triglycerides and glucose in the human subjects. Post-heparin LPL activity increased from 79 +/- 6.4 mU mL^{-1} in the fed state to 112 +/- 10 mU mL^{-1} in the fasted state ($P < 0.01$), while LPL mass was 361 +/- 29 in the fed state and 383 +/- 28 in the fasted state, respectively ($P = 0.6$). In contrast, fasting of guinea pigs caused an 80% drop in post-heparin LPL activity. The effect of fasting on human and guinea pig post-heparin HL activity were moderate and statistically not significant. CONCLUSIONS: In animal models such as rats and guinea pigs, post-heparin LPL activity decreases on fasting, presumably due to down-regulation of adipose tissue LPL. In humans, fasting caused increased post-heparin LPL activity.